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MICROBIOLOGICAL INDICATORS OF STERILIZATION. GENERAL PRINCIPLES

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16. Abstract A survey is presented of general properties of microbial indicators of sterilization and of principles involved in testing sterilization procedures with such indicators. It is suggested that the resistance of microbiological indicators should not longer be characterized by duration of action of the noxious agent nor effective killing dose. Probability theory shows it to be preferable to characterize the resistance of microbial indicators by the duration of action or dose of the agent which causes either a 50% or 99% reduction of indicators showing viable organisms. The logarithmic shape of the death curves, limited efficacy of sterilization procedures and unknown resistance of adhering organisms make it impossible to achieve absolute "sterilization," by definition a freeing of the object of all viable organisms. Sterilization only yields an object which, with a certain probability (generally very high), does not harbor viable organisms which might constitute a risk. Different indicators, showing ability of procedure to effect killing, are used for different objects and procedures. Indicator and sterility tests complement each other, but are not interchangeable.

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MICROBIOLOGICAL INDICATORS OF STERILIZATION. GENERAL PRINCIPLES

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Summary /528*

A survey is presented on the characteristic of microbiological sterilization indicators, as well as on the principles of testing of sterilization method by means of microbiological indicators. Based on the theory of probability, a comparison is made between the differing significance of the testing of sterilization processes with microbiological indicators and testing sterilized goods to determine sterility. It is recommended that the resistance of microbiological indicators no longer be characterized by the duration of action or dose of an agent employed to cause the absence of evidence for any surviving organisms. It appears preferable to characterize the resistance of microbiological indicators of sterilization by that duration of action or by that dose of the agent which causes either a 50% or a 99% reduction of indicators showing any viable organisms. The peculiarities of the testing of steam, hot-air, ethylene oxide and radiation sterilization with microbiological sterilization indicators are also discussed. The presentation leads to the conclusion that the sterilization methods are often unable in practice to fulfill the requirement that all viable organisms be removed from the object being sterilized. Sterilization results only in objects which, with a certain -- generally very high -- probability, do not harbor any viable organisms which might constitute a risk while the object is being used for the purpose for which it was designed.

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The seventh edition of the German Drug Book defines the term "sterilize" as a measure designed to free an object from all viable organisms. This definition contains a categorical requirement, which allows for no exceptions. The object must be freed of <u>all</u> viable organisms. The word "free" as used in this definition can be interpreted in the widest sense. The viable organisms must not be removed physically from the object by the sterilization process. The definition is fulfilled when they are no longer able to reproduce as a result of the treatment. Their "corpses" can remain on or in the object in question.

The goal of this paper is to sketch the rule and law valid for the testing of sterilization methods with microbiological indicators. This includes both classical (hot-air and steam sterilization) and modern (ethylene oxide and radiation sterilization) methods. It will become clear that both groups of methods ultimately have the same problems, only that these problems are differently accentuated. This knowledge is probably the key to a more professional evaluation of the utility of all methods, including the most modern.

General Properties of Microbiological Indicators

Microbiological indicators are understood here to mean objects to which microorganisms are adhering. These objects with the goods to be sterilized are subjected to the sterilization process and then tested to determine if organisms capable of reproduction are still present on or in them. The microbiological indicators are therefore employed to determine whether the process has achieved a certain effect on an object with known properties. The scale for this effect is the damage inflicted on the bacteria with regard to their reproduction capability under certain culture conditions. The indicator must respond to all factors, which contribute in the process employed to the

biologically measurable effect, as well as to all parameters, which /529
impair the development of effectiveness of these factors. Since, on the one hand, the various sterilization processes differ in the nature of the factors and parameters, and, on the other hand, one and the same type of bacteria does not exhibit a sufficiently high resistance to all processes, the ideal state that one and the same indicator can be applied in the testing of all processes will hardly be able to be verified.

The biological indicator shows the total effect, which results from the simultaneous or successive action of the individual factors, which can be substituted for one another. The biological indicator does not, therefore, yield information regarding the constancy of the conditions in the sterilization chamber, but rather only provides indication regarding the constancy of a single effect. Process technology must assure that the possibility of reciprocal substitution of the factors is only used and can only be used to a limited -- i.e., acceptable -- degree.

In the age of measurement and control technology, it must be startling at first glance to ascertain that both physical and biological methods are utilized in the testing of sterilization processes. The reasons for this are based on the requirements placed on the measurement system:

1. The measurement system must be small, so that the conditions on an almost punctiform measurement site can be recorded.

2. The measurement system must not influence the conditions prevailing at the punctiform measurement site.

3. The measurement system must be suited to the registration of conditions in small, hermetically sealed volume without being in direct contact with the surroundings.

4. The measurement system must not report the condition prevailing at the measurement site, but rather it must register the effects integral throughout the time of operation.

On the other hand, the greatest problem encountered is that the measurement system must be inexpensive, so that every sterilization apparatus can be equipped with a number of such systems. It is sufficiently well-known in practice that the conditions inside the goods to be sterilized show changes during the sterilization process, which are anything but uniform, and that it is not sufficient to record the effect integral at only a single point.

The requirements placed on these systems can only be fulfilled by the biological indicators at present, and it is expected that this will remain true for a considerable period of time. In addition, only these indicators make possible an objective comparison of the efficiency of various processes. We do not deny here that the microbiological measurement systems have several significant disadvantages when compared with physical systems:

1. inertia. The measurement result is not available until 24 hours later, at the earliest. In some cases, it does not become available for 14 days.

2. instability. The indicators have a limited use span -- a maximum of 1 year.

3. high costs. The manufacture, testing and investigation of the indicators is relatively expensive due to the high salaries paid personnel involved in such activities.

On the other hand, the biological test objects are not irreplaceable at any price. Their significance is dependent on the number and type of factors, which participate in the killing of organisms, and on the heterogeneity of the conditions in the sterilization chamber and in the objects to be sterilized. Microbiological indicators can most readily be dispensed with in the radiation sterilization process. If radiation sterilization represents one extreme, ethylene oxide sterilization represents the other extreme. In the latter process, it is certain that biological indicators are an absolute requirement.

Although the physical measurement systems do not fulfill the requirements presented here, they undoubtedly comprise some of the most important and necessary components of a sterilizer, since they are the only components with the capability of keeping the conditions in the sterilization chamber sufficiently constant, or regulating these conditions in the required manner during the sterilization process.

The Ratio Between the Duration of Effects of a Noxious Agent and the Number of Surviving Organisms /530

The microorganisms on an indicator do not die instantly due to the effects of a noxious agent, even when a pure culture is present. In the ideal case, which can often be encountered in practice, the number of viable organisms is reduced by a constant percentage per unit of time. The conditions in this case are analogous to those prevailing in a reaction of the first order. If the logarithm of the number of surviving organisms (log N) is plotted against the time (t) in a coordinate system, a straight line is the result (see Fig. 1). Based on the nature of the active agent, its concentration and the temperature, as well as the type and condition of the bacterial cells, these lines exhibit inclines, which differ in steepness. Certain concepts and characteristic magnitudes have become customary for the quantitative description of resistance capability of a type of bacteria or a bacterial population. Historically, these terms were developed to correspond to the course of thermal processes, but they can be applied in the same sense for other sterilization processes. They are illustrated based on Fig. 1 to the extent that they are of significance to the present paper:

The D-value (decimal killing time) gives the time in minutes, within which the number of viable organisms is reduced to 1/10. It represents a scale for the steepness of the survival curve.

The inactivation factor (IF) relates the size of the numerical ratio between the initial number of organisms (N_0) and the number of surviving organisms at a certain point in time during or after the action of the noxious agent (N). It is given as the logarithm of this quotient. In order to be able to characterize prolonged effects durations with an inactivation factor, the survival curve is extrapolated past log N = 0 when necessary.

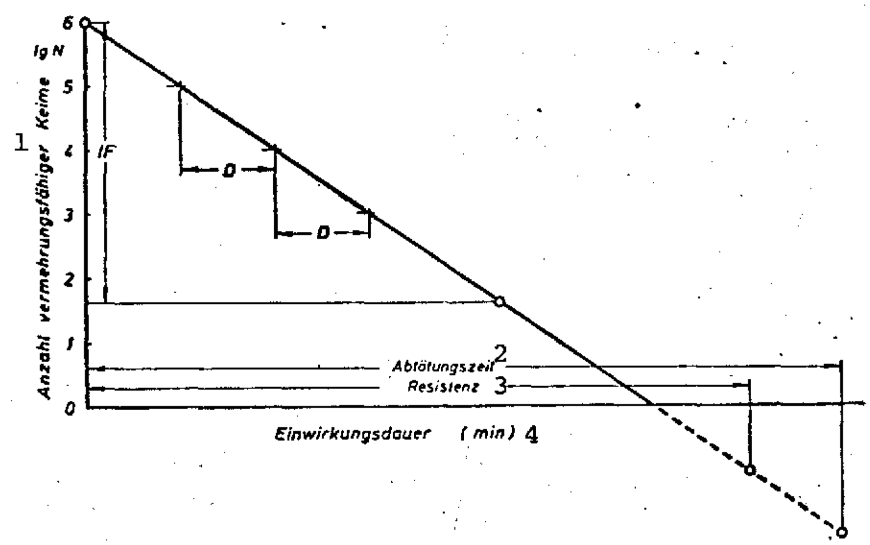

Fig. 1. Schematic depiction of a death curve in a coordinate system with the effects duration of the noxious agent as the abscissa and the number of viable organisms (log N) as the ordinate.

Key: 1. Number of viable organisms
2. Killing time
3. Resistance
4. Duration of effects (min)

Resistance is understood as the capability of a microbe population to form vegetative cells of the same type following the action of a certain (defined according to type, temperature, concentration, duration of action, etc.) damaging agent. The scale for this resistance is the maximum value of that factor, which was varied during the experimental determination. This is usually the duration of action. /531

The killing time provides the length of time a noxious agent must act until no viable organisms can be determined with the microbiological test method on the contaminated object or on the indicator. As was true of resistance, it is dependent not only on the biological properties of the cell, but also on the medium on or in which the cells are located.

We have already pointed out briefly the significance of the N-values located below the abscissa axis. The abscissa axis itself is located on the level log N = 0. In other words, only a single cell is surviving on the object under study. Since the cell is the smallest viable unit, the negative ordinate values shown in the extensions of the death curve above the abscissa axis cannot be considered to be viable fractions of a cell. These values must be interpreted from the standpoint of population statistics. Log N = 0 (or N = 1) then means that each of the units investigated contains an average of one viable cell. At log N = 1, one of ten units studied contains a cell capable of reproduction. At log N = 2, two of 100 units contains a cell capable or reproduction, etc. This leads to several important consequences for sterilization and biological testing of sterilization processes:

As long as the duration of action is not infinitely long, there is always a certain -- if very slight -- possibility that the object treated still contains a viable organism. The definition of the term "sterilization" given at the outset cannot, therefore, be fulfilled.

Even when our knowledge of the effects conditions is comprehensive, we can only predict the size of a portion of the population, which will have been killed by a certain point in time. The fate of a single organism remains uncertain, and the exact point in time of its death cannot be predicted.

The killing time becomes larger, the higher the initial number of organisms and the flatter the course of the death curve.

A small number of organisms of a resistant type (death curve with a flat course) can have a longer killing time than a large number of sensitive organisms (death curve with a steep course).

The Dependability of Sterility Testing and Indicator Testing

The efficacy of sterilization measures can be checked with two different microbiological methods -- sterility testing and indicator testing. Sterility testing is understood to mean the direct microbiological investigtaion of the sterilized products to determine their freedom from organisms. It is the more realistic test method, in that it provides information on the actual status of the sterilized goods, but the information received is not as definite as it might appear based on the facts presented. Since, as a rule, the entire charge cannot be checked during sterility testing (the material tested to determined sterility cannot be used again and, if the entire charge were tested, no material would be left for the intended application), but rather /532 the condition of a sample must be extrapolated to represent the condition of the entire charge, a large uncertainty from the statistical standpoint is presented by this method. The results are definitive -- with certain reservations -- only when the sampling uncovers a nonsterile object. In this case, the entire charge is declared to be nonsterile. If all samples from a charge are found to be sterile, this does not mean that the entire charge is sterile. The relationship between the degree of contamination of the charge, the extent of random sampling and the probability that only organism-free objects will be tested via this sampling procedure is depicted graphically in Fig. 2.

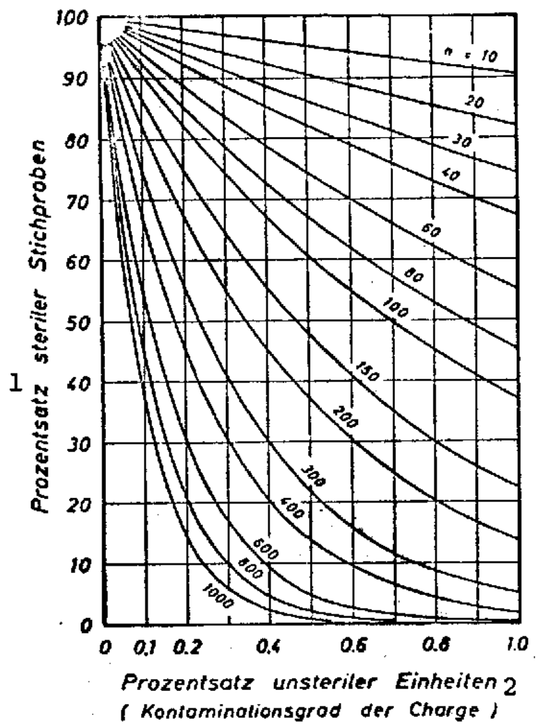

Fig. 2. Dependence of the frequency of sterile random samples (in %) on the degree of contamination of the charge (in %) and the number of units (n) tested to determine their sterility.

Key: 1. Percentage of sterile random samples; 2. Percentage of nonsterile units (degree of contamination of the charge).

When, for example, a charge contains 1% nonsterile components and a random sampling of 10 components is made, the probability that the sampling will contain only sterile units is 90% (this calculation is based on Poisson's distribution). If [illegible] components are tested, the contamination of the charge is not detected in 82% of the samples. Even when 100 samples are tested each time, the charge wil be falsely declared to be sterile in 37% of the cases. As can be seen in the graphic depiction, the frequency of "false determinations" increases strongly as the degree of contamination drops.

Accordingly, the sterilization test can only detect relatively high degrees of contamination. Even if economic considerations are not adhered to, this disadvantage can only be compensated for to a certain degree by increasing the number of random samples. When the sample is introduced into the culture medium, the danger exists that the sample will undergo microbial contamination caused by instruments or dust. The more extensive the random sample, and the more components are investigated, the greater the possibility that a sterile charge will appear to be nonsterile due to a foreign infection caused in the conduct of the sterility test. At a contamination frequency of 0.1% and a random sampling composed of 100 units, 9% of the random samples will contain at least a portion contamined via foreign infection. When the sterilization process is regulated properly, the sterility test gives better information on the contamination frequency in the testing laboratory than on the sterility of the product tested. /533

The efficacy of the sterility tests is also limited by the fact that it is not known which organism is present on the object and what growth conditions are required, in order to determine this organism. The usual quantity of 2-3 different substrates at 2-3 different incubation temperatures can only register a limited spectrum of microorganisms. This problem occurs repeatedly in the so-called "slow-grower" phenomenon, in that individual batches from the sterility test are rendered turbid by the growth of microorganisms long after the normal observation time has ended.

Under such unsatisfactory conditions, the value of the sterility test is questionable. For the reasons mentioned -- but not limited to those listed -- it is completely unsuited to the control of a sterilization process or of the conditions prevailing in a sterilization chamber. It can provide information on whether certain conditions prevailing in the initial state of the goods to be sterilized were not considered during the establishment of the sterilization conditions, whether the necessary requirements were not fulfilled or whether the goods were contamined subsequent to sterilization. We see, therefore, that the sterility test does not eliminate the need for the indicator test, although it is not absolutely needed when biological indicators are utilized. Although it is generally done today, it is in no case proper to use the sterility test alone or to consider the term "tested for sterility" as an absolute. The term "sterile" can in any case only be applicable to the individual object or to the sample taken during the sterility test. Strictly speaking, this term cannot be applied to the remaining portions of the charge.

In contrast to the sterility test, the indicator test has the advantage that the organisms to be determined or their growth conditions are known. The following has great practical significance: If regulated properly, the indicator test allows us to follow the action of the sterilization process past the so-called killing time of the population present in the goods to be sterilized. To a certain extent, it allows us to observe a certain margin of safety.

The Relationship Between the Mean Number of Surviving Organisms and the Frequency of Sterile Test Objects

Microbiological sterilization indicators can be studied to determine surviving organisms in two different ways -- qualitatively or quantitatively. In the quantitative process, the number of surviving organisms per object is determined, whereas the /534
qualitative process -- the so-called end-point method -- only determines if viable organisms are still present on the indicator. In principle, the end-point method is a sterility test of a conventional nature, for which the same rules of probability calculation are valid! The widely held opinion that all test objects become sterile simultaneously after passing a certain duration of action is false.

The conditions are best interpreted when the survival curve of the organisms on the test object and the probability curve for sterile or nonsterile test objects are presented above the

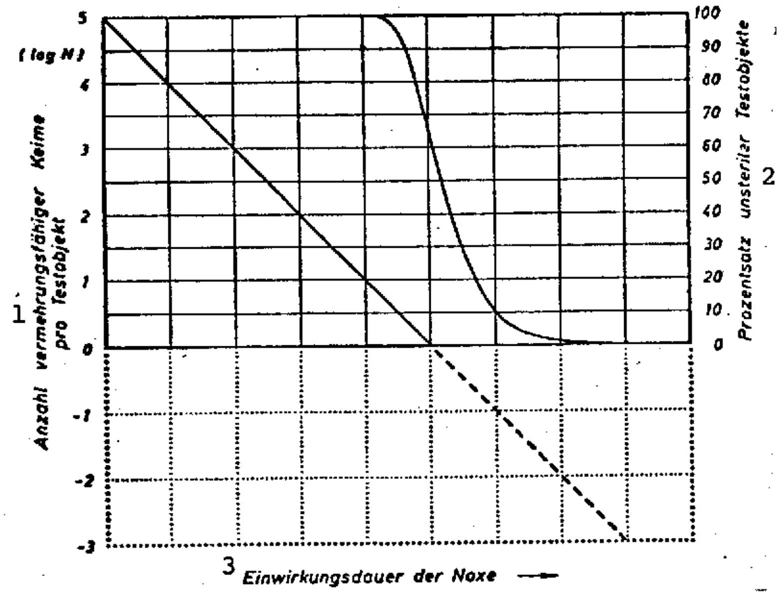

Fig. 3. Mean number of viable organisms per test object (left curve) and frequency of nonsterile test objects (right curve) dependent on the duration of action of a noxious agent.

Key: 1. Number of viable organisms per test object; 2. Percentage of nonsterile test objects; 3. Duration of action of the noxious agent.

same abscissa (see Fig. 3; the left ordinate scale is valid for the straight-line survival curve, while the right scale is valid for the S-shaped probability curve):

At short durations of action, all indicators in the microbiological test show growth. Even if the organism content falls below 10 per test object (log n = 1), the portion of sterile test objects increases rapidly. At a medium organism content of N = 0.7 (e.g., log N = -0.16), 50% of the test objects are sterile. In the further course of sterilization, the portion of nonsterile test objects only decreases slowly. At a mean organism content of N = 0.1, 10% of the test objects are nonsterile, while approximately 1% of the test objects are nonsterile at N = 0.001, etc.

Accordingly, we cannot expect all samples to be sterile theoretically until an infinitely long duration of effects of the organism-killing agent has passed.

In the previously used method to determine the characteristic killing time or the resistance of the indicators, a certain, generally small number of test objects was exposed to the sterilization process until all were sterile. The duration of effects necessary to accomplish this purpose was considered to equal the killing time. In complete contrast to its name, this process does not yield a precise end point. The reasons for this are based on random-occurrence statistics. From experiment to experiment, the killing time shows various values, and it is dependent simultaneously on the (not-standardized) number of indicators tested (see Fig. 4).

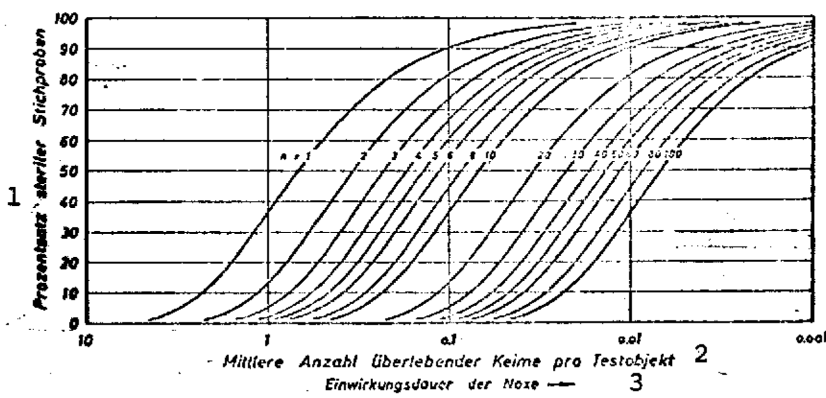

Fig. 4. Frequency of sterile random samples (in %) dependent on the mean number of surviving organims per test object and the size of the random sample or the number (n) of test objects investigated.

Key: 1. Percentage of sterile random samples
2. Mean number of surviving organisms per test object
3. Duration of action of the noxious agent

If, for example, only a single indicator strip is examined, it is sterile with a probability of 90% when the mean organism content is N = 0.1. If two indicator strips are tested, a 90% probability that both strips are sterile does not exist until the mean organism content is N = 0.053 (meaning only after a prolonged duration of action of the noxious agent). The requirement that five test objects must be sterile would not be fulfilled with the same probability until N = 0.021, etc. The more precise the so-called killing time is to be determined, the greater is its numerical value.

Utilization of the knowledge obtained from the probability calculation allows for a more precise description of the so-called resistance of microbiological indicators than was possible previously. This knowledge refers to the duration of action required to render a certain percentage (the most practical value being 50%) of the test objects sterile, and to the gradient of the inflectional tangent.

As shown by Fig. 5, 50% of the test objects are sterile independent of the course of the survival curve when the mean organism content log N = -0.16. Fig. 5 also shows that the presentation of this point in time is not sufficient by itself to describe the resistance of a population. Depending on the steepness of the survival curve, the probability that nonsterile test objects will be detected decreases with varying degrees of rapidity as the duration of action progresses. Since the steepness of the survival curve has a direct relationship to the steepness of the inflectional tangent of the S-shaped frequency curve, it can be employed for the quantitative description of resistance.

This leads to the following conclusions for laboratory /536
practice:

The percentage of sterile indicators must be determined as precisely as possible for several durations of action. In contrast to the technique employed previously, the number of test objects studied must be greatly increased. It must not be less than 50 for each duration of action investigated. On the other hand, the emphasis on the measuring points must not be placed unilaterally on that portion of the frequency curve with an asymptotic course, but rather the points must be equally distributed on both sides of the 50% value.

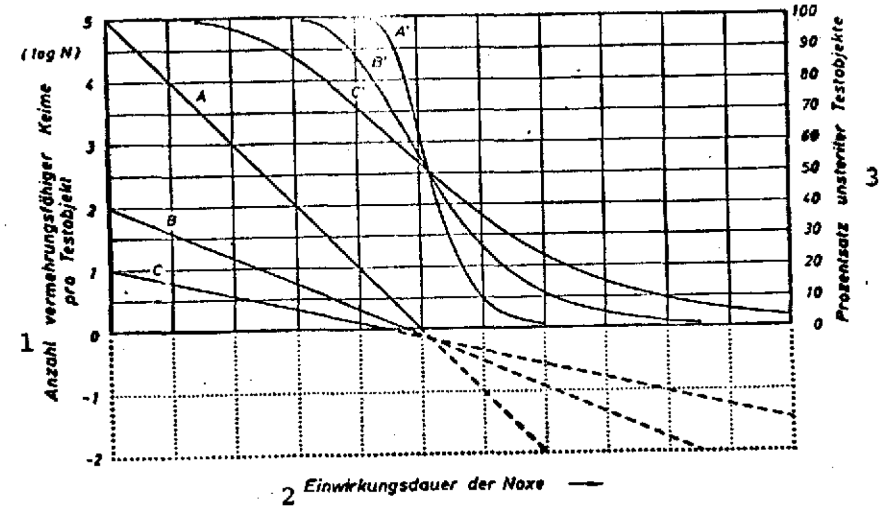

Fig. 5. Dependence of the course of the frequency curve of nonsterile test objects (right family of curves: S-shaped) on the steepness of the corresponding death curve of the test organisms (left family of curves: straight lines). The frequency curve A' corresponds to the death curve A. The frequency curve B' corresponds to the death curve B, etc.

Key: 1. Number of viable organisms per test object
2. Percentage of nonsterile test objects
3. Duration of action of the noxious agent

If the percentage values found for durations of action of various lengths are plotted onto a suitable probability network, a straight line will result in the ideal case. Interpolation can then be employed to determine that duration of action at which 50% of the indicators are sterile.

As has been pointed out previously, information on the steepness of the inflectional tangent or the straight lines obtained with the aid of the probability network is required for the characterization of the biological indicators. Since the position of a straight line in a two-dimensional coordinate system can be determined precisely with two points, it is sufficient to give a second time point in addition to the duration of action, which corresponds to the 50% value. The killing time, which has been in use for decades, can be employed for this purpose, although it must be provided with a definition better founded in mathematics. It is probably no longer that duration of action, at which "all" indicators are sterile, but rather at which a certain, sufficient percentage of the indicators is sterile. The size of this percentage value is only a question of agreement. A percentage of 99% sterile and 1.0% nonsterile objects is probably suitable in practice. Furthermore, the corresponding duration of action is probably not too far removed from the killing time according to the older definitions. Its numerical value can be determined via /537 extrapolation of the curve contained in the probability network.

If the biological indicators are studied with the end-point method, the sterilization processes can only be investigated to determine if a certain minimum effect is guaranteed. This minimum is that effect required to make all indicators (almost) sterile. It cannot be determined whether, and to what extent, the necessary value is exceeded. This represents a great disadvantage of the end-point process, which is the price paid for a simple analysis process. A quantitative microbiological test method is required if the sterilization process is to be investigated to determine the size of the effect at the individual measurement sites or to determine the degree to which the required minimum effect was exceeded. A requirement for this is that viable organisms in sufficient quantity are still present on the indicators after the effect required to sterilize the object in question has been exceeded. Under these conditions, contaminations occurring during the processing of the test objects are not as disruptive and do not have such serious consequences as they do when the end-point method is utilized. A single bacterial cell penetrating the individual charge during the counting of the organisms present would not impair the results of the experiment. In practice, it has strangely not been usual in the past to investigate microbiological indicators following sterilization to determine the number of surviving organisms. It is obvious that great difficulties exist in accepting and understanding the concept that a product can be completely sterile (in the sense of the present definition) if surviving organisms are still present on the indicator.

The Resistance or Killing Time of the Microbiological Sterilization Indicators

Fundamentally, the killing time of a sterilization indicator must be as long as that of the duration of action required for the sterilization task at hand. During the preparation of the biological indicators, therefore, the following question is posed again and again: Which duration of action or what dose must the indicator show? If we require that the sterilization process must yield a sterile product always and under the worst possible conditions, hardly any problems would be encountered in the answering of this question. The best test object in such a case would certainly be samples of native soil. Packages of soil have been used for many years as sterilization indicators in Germany, although this by no means indicates that the maximum requirements have actually been fulfilled. The soil samples are by no means sterile, even when they show no growth after incubation at 37°C for 14 days in bouillon. When the investigation techniques are modified, surviving organisms can always be determined. If we should insist on the fulfillment of the maximum requirement, the damage would exceed the utility of such processes. If the product is sterile but unusable, the sterilization robs itself of its purposes. If the process yields assured results, but is too expensive to be economically feasible, it lacks the most important condition for utility. If a sterilization method is discarded

only because it cannot fulfill a task, with which it is not confronted in actual practice, the testing method is not sensible. It should also be remembered that processes, which do not fulfill the maximum requirement -- and this includes the classical sterilization process using steam under pressure -- have been proven under actual conditions for almost a century. We /538
shall now present a brief discussion of the most important parameters, which must be considered in the determination of the duration of effects or doses to be required:

Type of organism. The rate at which the number of viable organisms of a population decreases under the influence of a noxious agent is dependent on the nature of the organism. Both sensitive and insensitive genuses and species exist. At the same time, no correlation exists in the resistance capability toward various agents. An organism resistant to high-pressure steam can be sensitive to hot air, while a heat-sensitive organism can be insensitive to gamma radiation.

Development state of the organism: In general, bacterial spores are more resistant than vegetative cells. This rule does not apply to radiation sterilization and obviously not to ethylene oxide sterilization. The resistance capability is also dependent on the life conditions, under which the organisms have developed.

Number of organisms: The more organism present on an object to be sterilized, the longer a sterilization process must be allowed to act to achieve the desired low organism content.

External factors: The efficacy of an organism-killing agent can be impaired to a large degree by substances covering the organisms. This effect is especially disruptive during ethylene oxide sterilization. The resistance capability of the organisms is also dependent on microclimatic conditions, especially relative humidity and partial pressure, as well as the nature of the gases.

The so-called resistance of a population is based on these four parameters. The duration of effects required to overcome this resistance can be determined experimentally on representative random samples of the "naturally" contaminated goods. This is represented by that duration of action, following which the objects are found to be sterile in a sterility test. Naturally, it has a close relationship to the organism content of the raw material, the preparation process, the hygiene observed in the preparation tecynique and the preparation of the goods for sterilization (cleaning). Since with few exceptions it is impossible to include the worst possible cases during the estimating of the required duration of action (or temperature, concentration or dose) of the organism-killing agent, the opposite tack must be taken and the worst possible case must be eliminated prior to the beginning of the sterilization process.

The duration of action required to generate a product that is shown to be sterile in the sterility test must be increased by the so-called safety span (see Fig. 6). The following points must be considered during the estimation of the safety span:

steepness of the survival curve: The flatter the survival curve of the contaminants, the slower the decrease in the organism content during the course of sterilization and the later a small organism course is achieved.

type, application purpose and value of the object: If the possibility exists that the surviving organism can reproduce in or on the object to be sterilized, the safety factor must generally be greater (e.g., longer) than in those cases in which the number of surviving organisms cannot increase (instruments, devices).

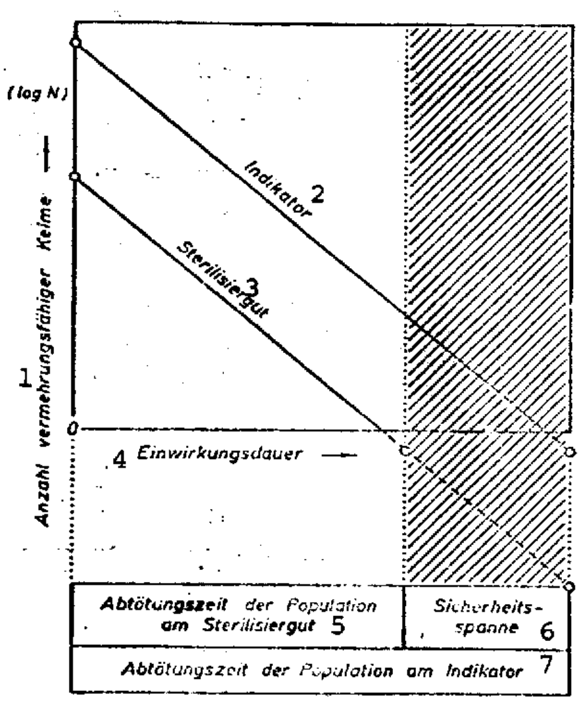

Fig. 6. Schematized graphic depiction of the death curve of the organism population in the goods sterilized and on the indicator, as well as the safety span.

Key: 1. Number of viable organisms; 2. Indicator; 3. Goods to be sterilized; 4. Duration of action; 5. Killing time of the population on the goods to be sterilized; 6. Safety span; 7. Killing time of the population on the indicator.

In this case, the conditions of storage (temperature, duration) also play a role. The certainty with which the consumer can recognize the spoilage of the product must also be considered. Dependent on the application purpose of the object, certain types of organisms can be disregarded, such as is the practice in medicine with the spores of extremely thermoresistant, thermophilic bacilli. In scientific microbiology, it is certain that higher requirements than those adhered to in medicine must be followed than is presently the case in many instances. It is paradoxical, for example, that NASA utilizes steriliza- /539
tion procedures on spacecraft to prevent contamination of celestial bodies, which "only" fulfill medical requirements. The value of the object must also be considered. In the case of sensitive electronic devices, the duration of action or the dose is generally calculated somewhat smaller than in consumer goods. On the other hand, even the latter type of object must not be exposed to damage during sterilization, which exceeds acceptable limits.

economy of the process: In cases in which it is possible to select as high an effects intensity as desired, economic reasons often dictate that this not be done. This is true of the sterilization of instruments and devices in medicine. The expenditure considered necessary must always be able to be justified.

risk: The risk, which the producer is willing to take or which the consumer can be expected to take, is very important in the selection of the safety span. Consequences resulting from use of a nonsterile object can vary widely from case to case. The safety span ranges from a single spoiled package to the death of one or more persons.

The safety span must be even larger in proportion to the inhomogeneity of the starting conditions and the more inhomogeneous the contamination of the goods to be sterilized. The aforementioned parameters must be assigned various weights in the determination of the required duration of action depending on the process and goals at hand. In general, the safety span should be as large as possible. Due to the wide differences in processes and the manifold nature of the tasks assigned, we cannot expect that a single indicator type will be applicable for all purposes. This leads to the obligation to know precisely the /540
application range and to maintain the conditions upon which the estimates were made -- namely, the condition of the goods to be sterilized prior to sterilization -- or to control the maintenance of these conditions in a suitable manner.

Natural Organism Populations As Sterilization Indicators

Parts of the goods to be sterilized cannot be employed as sterilization indicators. On the one hand, the initial organism count and therewith also the killing time of the population would not be constant. On the other hand, this would not allow for the maintenance of a safety span. Even if the contamination of these objects were known to be more intense than usual, the safety span as calculated would be too short. This peculiarity results from the logarithmic order of death: If 10^2 organisms are already present, a doubling or tripling of the organism count does not increase the killing time by much. We are left with no choices but to seek other, suitable natural objects contaminated in the same form or to contaminate suitable objects in the same form. The objects to be used for this purpose must not necessarily be similar to the goods to be sterilized. It is primarily important that, under the conditions of the sterilization process, the resistance of the population on the object must have a certain relationship to the organisms on the object to be sterilized.

The most important natural sterilization indicator is so-called spore soil. This is nothing other than sieved native soil -- usually composted soil -- packaged in quantities of about 0.2-2.0 g. This "spore soil" must not -- and we emphasize this point -- be considered to be a model for the normal impurities of the goods to be sterilized, but is only representative of a substrate containing a population with the required resistance. Following sterilization, it must not be investigated to determine its sterility with the methods listed in the Drug Book, but rather only to determine if organisms have survived, which can reproduce within 14 days in bouillon at 37°C. In those cases where the task actually exists to sterilize soil, the normal spore soil is unsuitable as an indicator due to the lack of a safety span. It can serve in this case only as a test object to determine the resistance or the killing time of the population present in the earth.

Repeated objections have been made to the use of native soil as a biological sterilization indicator -- especially by Anglo-Saxon authors. In my opinion, these objects are not valid, otherwise the experimental results would show intense fluctuations. These authors usually do not consider that the spread in the findings represents a statistically caused peculiarity of the end-point method. This will always be a problem when the end-point method is used -- regardless of whether we are dealing with soil samples or filter paper strips, which have been impregnated with pure cultures. The soil disappointed these authors, since they obviously suspected that each soil would exhibit the same resistance, and that they could be used as indicators without testing them previously to determine resistance. It is certainly a great disadvantage that the resistance cannot be regulated in a soil sample, and that soils of various origins must be prepared and tested until a suitable type is found. Instead of damning this soil as a test object, research could be employed to find such a test material, which is very inexpensive and unsurpassed in the constancy of its resistance characteristics.

The resistance of the soil samples cannot, for the reasons /541 detailed above, be regulated to any noteworthy degree, but the conditions are probably not as bad as indicated by the experimental results presented by Österle (1937). It should not be overlooked that Österle mixed the soil samples graduated from 0.1 to 10 g with a constant quantity (10 ml) of bouillon, thereby exposing the smaller samples to entirely different heat-treatment conditions than the large samples were exposed to. An exact determination of the dependence of the resistance on the quantity of the soil would be desirable, in order that we might ascertain whether, and to what extent, the previously recommended quantity of 2 or 3 g can be reduced. In large-scale experimental series, the quantity required at the present time can cause procurement and preparation difficulties. For this reason, the advantage of resistance constancy cannot be utilized fully.

The disadvantage that native soil is a mixed culture could also be utilized as an advantage. The possibility to alter the spectrum of the determinable organisms and therewith the "apparent" resistance of the earth by changing the culture conditions -- both with regard to the substrate and the temperature, as well as the duration of incubation -- has never been utilized. This does have the disadvantage that the soil samples can only be used by that person, who knows the required culture conditions in each case, and therefore ultimately only by that person, who has determined the resistance. Basically, however, this does not represent an additional limitation. It is also true of the commerical indicators, which only exhibit the nominal value when they are tested under the same conditions on surviving organisms, which prevailed during the determination of their resistance.

Another advantage offered by the soil as a mixed culture has not been accorded sufficient attention in the past. The soil is the only test object, which can probably be used in the testing of all sterilization processes. It is improbable that a correlation exists between hot-air, steam, radiation and ethylene oxide resistance, but no quantitative data has been presented to datefrom comparative studies of soil samples of various origins.

Pure Cultures As Sterilization Indicators

Aside from natural populations, microbiological sterilization indicators are in use, which have been prepared with the aid of pure culture. These are organism suspensions dried onto filter paper, glass, sand or aluminum foil, partially in combination with a freeze-dried or ready-to-use culture medium, as well as with a chemical indicator. In several forms, the chemical indicator shows whether a certain organism-damaging noxious agent has acted on the test object. In other models, the chemical indicator is designed to facilitate the evaluation of the sterility test of the microbiological indicators. It shows a color change to indicate the growth of surviving organisms in the culture substrate. Several authors recommended spores suspended directly in the culture medium as biological indicators. The basic principle that the indicator must respond to all factors participating in the sterilization process is not fulfilled by all biological indicators described in the literature or available commercially.

Pure cultures offer the advantage that the resistance of the /542 test objects can be regulated more or less actively to the required resistance level by variation of the cultivation and preparation conditions. Knowledge of the external factors upon which the resistance of an organism are dependent is therefore doubly useful. On the one hand, it enables us to exclude highly resistant conditions from sterilization or to convert these conditions into less resistant ones prior to sterilization. On the other hand, it enables test objects with the required resistance to be manufactured. The possibilities are manifold. The scale extends from the selection of an organism carrier via the suspension medium and the degree of drying to the inclusion of the organism in crystals (Doyle and Ernst, 1967). The composition of the substrate, which is employed to determine surviving organisms, is also very important in this instance.

Pure cultures make it possible to determine quantitatively the intensity of a sterilization process via organism counts. The survival curve determined in special experiments assumes the function of a calibration curve in this case. The efficacy of a process can be calculated from the ratio between the initial organism count and the number of surviving organisms, although this method is never used in practice.

Organisms must be used in the preparation of biological sterilization indicators, which do not place any special demands on the microbiological determination process. Accordingly, anaerobic organisms are less suitable than aerobic organisms. Of the latter, those types are preferred which can grow in simple substrates. During the selection of a strain, care must be taken to determine if the resistance is constant, both from charge to charge and during a 12-month period of storage. Pure cultures have almost always been disappointing with regard to resistance constancy. It is questionable whether they can compete with native soil. Aside from the aforementioned requirements, which are almost self-understood, the bacterial strain must be readily identifiable. It should be able to be differentiated from the normal contaminants occurring in a testing laboratory by a special characteristic.

No correlation exists in the microorganisms between the relative resistances to hot air, high-pressure steam, ionizing radiation and ethylene oxide. Pure cultures therefore have the advantage that one and the same strain need not be used for the various bactericidal agents.

Steam Sterilization and Microbiological Indicators

In the steam sterilization process, the object to be sterilized is heated in a steam-saturated atmosphere. The steam must be free of air. The reasons for this are not clearly stated in the literature. It is probable that the following reasons apply:

1. The heating through of porous goods is rendered more difficult or is inhibited when air is present.

2. When air is present, the correlation between temperature and pressure is changed, and the sterilization process cannot be regulated by a manometer.

3. An air-steam mixture has a smaller heat capacity than pure steam with the same temperature and pressure. This results in the fact that the capability to transmit heat to the goods to be sterilized is reduced.

4. Due to the differing specific gravities of air and steam, /543
a homogeneous gas mixture cannot be developed in the chamber.

5. Due to differing condensation temperatures of air and steam, the atmosphere enclosed in the sterilization chamber can undergo demixing.

The biological test objects in steam sterilization must both respond to the temperature and indicate the presence of steam. The property of the spores to be much more resistant to hot air than to steam with the same temperature can be utilized for this purpose. Since the indicators must show the presence of steam, they must make direct contact with the atmosphere prevailing in the sterilization chamber. The use of spore suspensions, which are enclosed into ampules by melting the ends shut, is usually against the rules or is at least acceptable only when the dependability of the sterilization process cannot be impaired by the admixture of air to the steam.

The D-values of the spores of the sensitive and resistant strains show behavior -- based on the same temperature -- with a ratio of 1:10,000 in the extreme cases. Despite the wide differences in the resistance of the spores, they are very similar in the dependence of the death time on the temperature. The death time increases almost fold when the temperature is changed by 10°C. This peculiarity is the absolute -- but usually unnoted -- condition that the biological indicator need only be calibrated at a single temperature, that it can be used at temperatures different from the calibration temperature, and that one and the same organism can be used as a sterilization indicator at various temperatures.

It is impossible in practice to operate steam sterilization processes in such a manner that the most resistant populations are also killed (see Fig. 7).

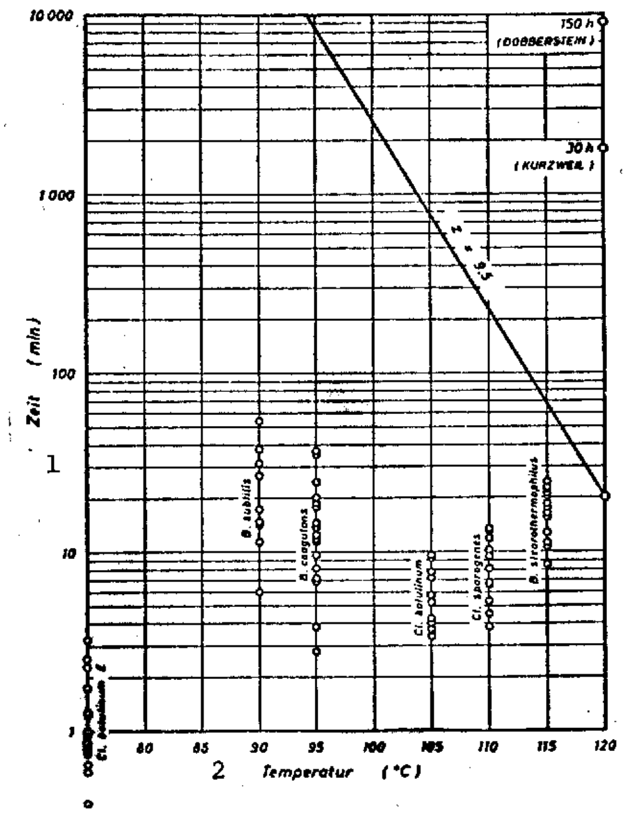

Fig. 7. Graphic depiction of D-values of the spores of Bacillus coagulans, Bacillus stearothermophilus, Bacillus subtilis, Clostridium botulinum and Clostridium sporogenes for high-pressure steam. The death time calculated by Kurzweil (1954) and Dobberstein (1959) for thermophilic soil bacteria are also plotted in the diagram. The curve shows the average dependence of the D-values or the death time on the temperature. At 120°C, it equals the duration of action of 20 min, which is the period prescribed in the German Drug Book.

The D-values were obtained from the following publications: Amaha & Ordal (1957); Augustin & Pflug (1967); Briggs (1966); Cook & Brown (1964, 1965); Cook & Gilbert (1965, 1968, 1969); Davis & Williams (1948): Donnellan, Nags & Levinson (1964); El-Bisi & Ordal (1965a, b); Ley & Tallantire (1964): Licciardello & Nickerson (1963); Molin & Snygg (1967); Murrell & Scott (1966); Roberts & Ingram (1965); Stumbo (1965); Tsuii & Perkins (1962).

Key: 1. Time; 2. Temperature

Fig. 7 contains D-values calculated by various authors for several bacterial strains at certain temperature. The values are presented in graphic form. When the values were not already available for the temperature in question, they were calculated using a z-value of 9.5. This possibility was only used when the temperature deviated from that required in the graphic depiction by not more than 10°C. A straight line was drawn through the point 120°C-20 min, which are the conditions recommended in the Drug Book for steam sterilization. The ascent of this line corresponds to a z-value of 9.5 -- e.g., 10 times the D-value at a temperature reduction of 9.5°C. The greater the distance of the plotted D-values from this line -- measured parallel to the ordinate -- the larger the inactivation factor achieved under the standard sterilization conditions. For Clostridium sporogenes, for example, this factor equals at least $10^{220/14} = 10^{15.7}$, while it is $10^{66/26} = 10^{2.5}$ for Bacillus stearothermophilus.

Bacteria, which are as resistant as B. stearothermophilus, are either not affected or not completely destroyed by steam sterilization. The opinion prevails in medicine that such abnormally resistant organisms need not be considered, since they are apathogenic and grow very slowly at a temperature Of 37°C. Whether or not these arguments are completely valid in an era when immunosuppressive treatments are possible, is questionable. It is probable that the problem is solved by the fact that an-lege-artis prepared, especially cleaned objects do not contain any highly thermoresistant organisms, or the number of such organisms is so small that the conventional sterilizations suffice to eliminate them. The irrefutable argument that the normal guide- /544
line values in medicine have proven themselves for many yeards, might find a scientific explanation in this manner.

The sterilization guidelines usually employed in medicine can only be valid under certain conditions and cannot be used without reservations in other areas of science. An indication is often missing that these methods are by no means sufficient for certain other scientific investigations, and the lack of such an indication can lead us down false paths. For example, Kurzweil (1954) determined populations in soil samples, which re- /545
quired a killing time of 30 hours at 120°C. This time lies far above the standard line in Fig. 7. This was exceeded several years later. The resistance of 4 1/2 hours observed by Dobberstein (1957) at 134°C is equivalent to a resistance of 110 hours at 120°C.

The classical biological indicator of steam sterilization is native soil. The requirement placed on such soil by DIN 58947 appears to be insufficient from the points of view presented in the present paper. When only 1/3 of the sample needs to contain surviving organisms after 5 min at 120°C and, after 8 min, all packages should be "sterile," we must ask the question as to how we can test the 20 min of action of steam at 120°C required by the Drug Book.

Among the pure cultures, _B. stearothermophilus_ is practically predestined for the manufacture of indicators for steam sterilization due to its high resistance. Previously, difficulties existed with regard to the relatively slight constancy of the resistance during storage of the test objects. The cause of this low degree of storage stability could lie in the heat activation of the spores undertaken during the preparation of the suspension (Cook & Brown, 1965). A certain additional expenditure for the microbiological laboratory is involved in the use of _B. stearothermophilus_, since the cultures must be incubated at a temperature of 50-60°C.

Hot-Air Sterilization and Microbiological Indicators

The bacterial spores do not show the same large differences in resistance to hot air that they exhibit toward steam. Based on one and the same temperature, the D-values behave as 1:20. The killing times also show similar changes proportional to the temperature, although the factor is much smaller than in the case of steam sterilization. The temperature must be increased by an average of 24°C when the death time is to be reduced to 1/10. Fig. 8 represents a graphic depiction of several D-values. The straight line drawn in gives the mean temperature dependence of the D-values. It was positioned in the diagram in such a manner that it corresponds at 180°C to that time (30 min) recommended by the Drug Book as a duration of action for this temperature.

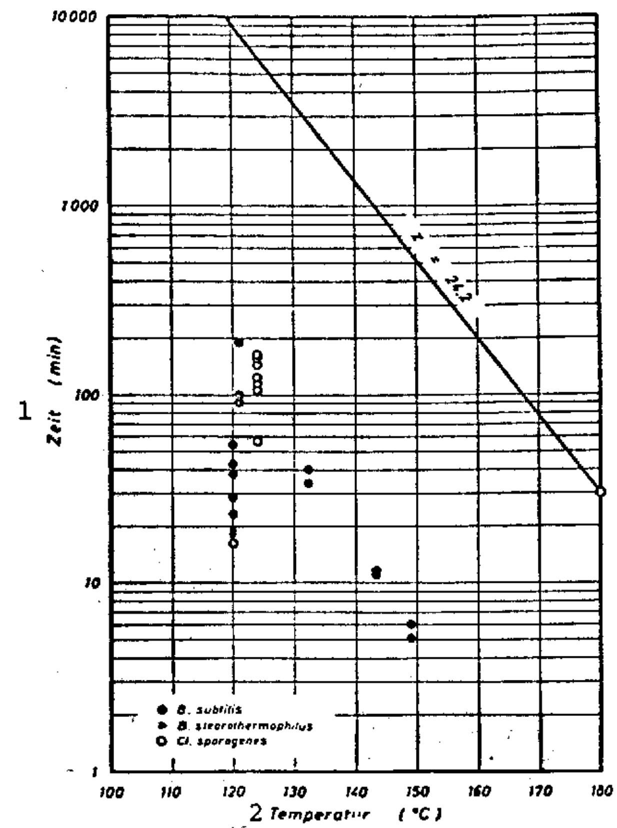

Fig. 8. Graphic presentation of the D-values of spores of Bacillus stearothermophilus and Clostridium sporogenes for hot air. The curve provides the average dependence of the D-values on the temperature. It equals the 30-min duration of action at 180°C prescribed by the German Drug Book.

The D-values were obtained from the following publications: Augustin & Pflug (1967); Koesterer & Bruch(1963); Doyle & Ernst (1967); Pheil, Pflus, Nicolas & Augustin (1967).

Key: 1. Time; 2. Temperature

Native soil is usually employed as a biological indicator in hot-air sterilization. The resistance of the soil is generally not tested with hot air. A type of soil is employed, which has been proven suitable for steam sterilization processes. The basic principle that the resistance of the biological indicators must be tested against the same agent -- to serve as a check on the effect of the agent -- is not guaranteed in this instance. This is even more surprising, since it has not been determined that a correlation exists in native soil between the resistances to steam and hot air. In this case, it is obvious that no differentiation has been made between the terms "indicator" and "model pollution." If native soil is actually to be rendered sterile by hot air, it must be exposed to a temperature of 180°C for about 60 min, which is approximately twice the time prescribed by the German Drug Book. Accordingly, hot air can be employed at acceptable expenditure levels to almost fulfill the definition of the term "sterilize." This represents the strength of the hot-air process. Unfortunately, both the high temperatures and the inertia involved in this process -- several hours are re- /546 quires before objects of large size can be heated to 180°C -- limit the applicability of this method. It is still questionable whether a hot-air resistance of 60 min at 180°C is actually the maximum possible. Systematic investigations of this problem must still be conducted.

Although it is obvious that one and the same native soil can be employed as an indicator in both steam and hot-air sterilization processes, this is not true of the pure cultures: B. stearothermophilus, the test bacteria of choice in steam sterilization, where is exhibits superior resistance properties, is not simultaneously suited to the testing of hot-air sterilization. Its resistance in the latter method is too small (see Fig. 8). Bacillus subtilis occupies the top position on the resistance scale. The variety B. globigii or B. subtilis var. niger can be differentiated /547 easily from other organisms based on its coloration. Based on theD-values found in the literature, the resistance of this strain is probably not quite sufficient to test the standard sterilization conditions in the hot-air process (180°C; 30 min).

Ethylene Oxide Sterilization and Microbiological Test Objects

The microbicidal effect of methods using ethylene oxide as an organism-killing agent is dependent on the ethylene oxide concentration, the pressure of the steam used, the temperature and the duration of action. The conditions are complicated and difficult to survey, because a large number of factors can influence the steam pressure and the ethylene oxide concentration at the site of action: diffusion barriers composed of organic and inorganic material, substances that absorb or hydrolize ethylene oxide, condensation of the components of the gas phase at the cold points, degree of swelling of the organism and its surroundings, direction of the steam pressure gardient in the vicinity of the organism, composition and structure of the organism carrier, etc.

Ethylene sterilization assumes a special position, because only slight resistance differences exist between vegetative cells and spores. No especially resistant strains or types exist. Even the highly thermoresistant organisms do not appear to represent exceptions to this rule. In this regard, ethylene oxide sterilization might be the ideal process of the prevailing difficulties inherent in this method can be overcome. The resistance of a population is basically dependent on the physicochemical state of the cells and their milieu. For this reason, no dependable data is available regarding ethylene oxide resistance of the organisms themselves. The values found in literature are largely falsified due to components of the cultivation medium, components of the suspension medium and insufficiencies of a process-technological nature. In addition, the fact that all organisms exhibit high ethylene-oxide resistance below a certain degree of swelling also renders difficult the determination of dependable data.

Whereas the survival curves in all other sterilization processes shows a straight course in a semilogarithmic coordinate system over several logarithmic cycles (although a certain starting phase is required before this line becomes straight), this is only true under optimum sterilization conditions when ethylene oxide is employed. The inactivation curve often moves along a horizontal course after an initial period of steep ascent. The percentage of resistant cells becomes larger, the lower the relative humidity of the ambient atmosphere to which is was exposed prior to ethylene oxide treatment, and the lower the steam pressure in the sterilization chamber. Intensely dried organisms lose their resistance very slowly in a steam-saturated atmosphere (Doyle & Ernst, 1968).

The insufficiency of the ethylene oxide process (Ernst & Doyle, 1968) can only be overcome, and biologicaly sterilization indicators can only be employed purposefully, when strict rgulations are followed in the construction of the apparatuses and the process technology employed. From the construction side, it must be assured that the chamber is filled with a gas mixture having a homogeneous composition. /548 Careful thermal insulation must be provided to prevent condensation or demixing of the gas (ethylene oxide, inert mixing gas, steam). Since a close relationship exists between relative humidity and temperature, local overheating must be avoided. Simultaneously, the problem of the heating of the goods to be sterilized must be solved. Prior to the onset of effect of the ethylene oxide, the conditions must be created to assure that the death curves show a logarithmic course throughout the entire diagram.

When testing ethylene oxide processes with biological indicators, it is not sufficient that these show a certain duration of action or concentration of the ethylene oxide, but rather they must simultaneously furnish information indicating the capability of the process to overcome certain difficulties.

Native soil shows varying resistances to ethylene oxide. Soil types are reputed to exist, which cannot be sterilized with ethylene oxide, whereas others provide no difficulties whatsoever. It is false to assume that Kayser & Liebermeister (1964) have provided a generally valid explanation for the high resistance of these soils. The resistance is based on an extractable, transmittable, water-soluble factor only in one particular case. The differences in the degree of ethylene oxide resistance shown by soils must not be overevaluated, however, at least until soil is not required to function as a contamination model. As was true in steam sterilization, a soil type could be selected in this case based on its resistance properties, or, as was recommended by Struppe (1969), the resistance of the test soil could be reduced to the required level via prior treatment.

On the one hand, it is disadvantageous that the resistance of the microorganisms to ethylene oxide is dependent to such a large degree on external factors. Simultaneously, it is advantageous to be able to regulate the resistance of the test objects using so many different methods (Adam, 1971; Beeby & Whitehouse, 1965; Doyle & Ernst, 1968; Coyle, McDaniel, West, Whitbourne & Ernst, 1970; Nyström, 1970; Royce & Bowler, 1961; Vales, 1971). The danger does exist that the resistance selected will be too-heavily adapted to the weaknesses of the process. The type and manner of preparation of the bacterial suspension and the selection and preparation of the organism carrier is more important in this case than the bacterial species and its development phase. By the addition of organic or inorganic substances to the suspension medium (Brewer & Arnsberger, 1966; Doyle, 1971) up to the inclusion of the organisms in (water-soluble) crystals (Doyle & Ernst, 1967; Mullican & Hoffman, 1968), any degree of difficulty can be regulated. It is noteworthy that the recommendation to employ infected sand as a test object meets with objections. A good criterion for the effective ethylene oxide processes is probably represented by the sterilization of freeze-dried preparations. In most instances, the test organism is *B. subtilis* var. niger, the same bacteria used in hot-air sterilization.

Since the ethylene-oxide resistance of the test objects depends to a large degree on the chemical purity of the bacterial suspension (Ernst & Doyle, 1968), all efforst to conduct valid biological testing of ethylene oxide sterilization processes cannot be compared until a standard method is developed to test the resistance of the test objects. The results published by various laboratories cannot be compared for this reason. The standard procedure must be specific with regard to the ethylene oxide concentration, steam pressure and temperature, as well as conditioning conditions of the test objects prior to the start of resistance testing.

Radiation Sterilization and Microbiological Indicators /549

Ionizing radiation shows the advantage of sterilization agents that the objects to be sterilized can be processed in gas-tight, closed containers at room temperature. If gamma rays are employed, a large depth effect represents an additional advantage.

Microorganisms also show various resistances to ionizing rays. In contrast to the thermal processes, the determination of the required radiation dose presents problems in the case of the vegetative organisms and not the spore-forming organisms. The radiation resistance of the organisms is dependent to a certain extent on the chemical composition and the water content of the milieu or the atmosphere, but not nearly to the degree observed in ethylene oxide sterilization.

Fig. 9 shows the death curves of several important organisms. The abscissa corresponds to the radiation dose, while the ordinate represents the number of surviving organisms. The effects spectra of the two radiation doses under discussion here -- 2.5 and 4.5 mrad -- are shown via shading. Bacterial spores generally show behavior similar to that of Bacillus subtilis. The survival curves show linear courses, and a dose of 2.5 mrad reduces the number of viable cells to at least 10^{-6}. When bacterial spores are employed, maximum radiation doses of 2.5 mrad can be tested. Micrococcus radiodurans and Streptococcus faecium are at present the microbes with the greatest radiation resistance. Their resistance appears to be based on a mechanism which is capable of repairing the damaged nucleic acid. Micrococcus radiodurans

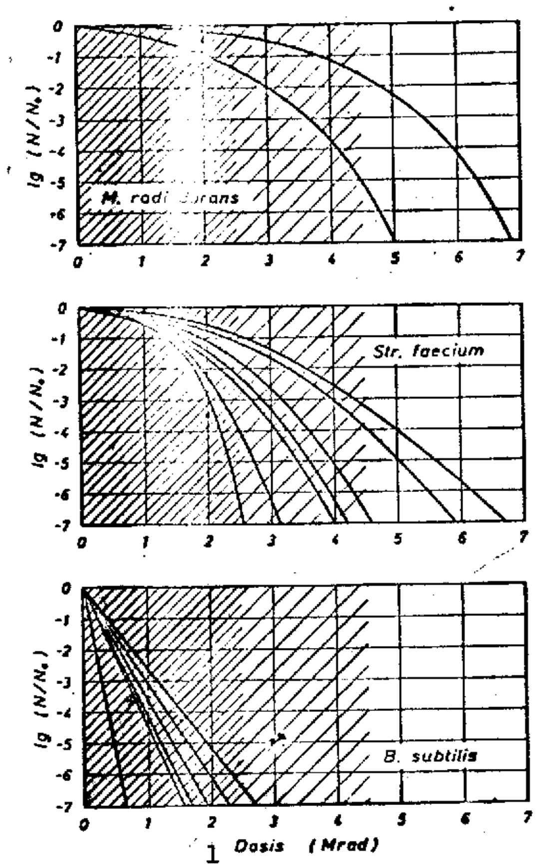

Fig. 9. Death curves of Micrococcus radiodurans, Streptococcus faecium and Bacillus subtilis exposed to ionizing radiation.

N_0 = number of viable organisms prior to action of radiation; N = number of viable organisms after the action of the radiation.

The death curves are based on the experimental results of the following authors: Christensen (1964); Christensen & Holm (1964); Christensen, Holm & Juul (1966); Christensen & Kjems (1965); Christensen & Sehestedt (1964).

Key: 1. Dose

was discovered in 1956 (Anderson, Nordan, Cain, Parrish, & Duggan). Streptococcus faecium was detected in 1919, but its radiation resistance was not generally known until 1964 (Christensen). It is probably only a question of time until further radiation-resistant genuses or species are discovered. The fact that the survival curves do not show linear courses, but do exhibit an extremely large shoulder, is characteristic of these highly resistant bacteria.

The discovery of the large radiation resistance of Streptococcus faecium led to the doubt that the sterilization dose of 2.5 mrad, which had been used on a worldwide basis for more than a decade, might not be sufficient. As long as only Micrococcus radiodurans was the only known radiation-resistant microorganism, its limited distribution could be used as an argument against an increase in the usual radiation dose. This argument is invalid with regard to the ubiquitous Streptococcus faecium.

According to the curves shown in Fig. 9, the number of viable Streptococcus faecium is reduced in the worst case to only 1/10 after a radiation dose of 2.5 mrad. In the case of Micrococcus radiodurans, the number is reduced to only 1/2. Even when we follow the recommendation of Christensen, Holdm & Juul (1966) and increase the sterilization dose to 4.5 mrad, very strict scales of organism reduction in the object to be sterilized will have to be adhered to. The limit must be so low that the radiation dose is sufficient to destroy completely the population contaminating the object, even when this population is composed of organisms as resistant as Streptococcus faecium. Even at a dose level of 4.5 mrad, the present state of the art does not allow that more than 10^2-10^3 viable organisms remain on the object. This tolerance is only valid if the presence of /550
Micrococcus radiodurans can be eliminated with certainty.

Very little is available concerning the suitability of native soil as an indicator for radiation sterilization. The resistance does not appear to exceed 2.5 mrad. The spores of B. pumilis are generally employed in the manufacture of "artificial" test objects. In most cases, no studies were conducted to determine just how high the resistance of such test objects really is. In most cases, however, it is probably far below 2.5 mrad. The cells of Micrococcus radiodurans and Streptococcus faecium possess a sufficiently high radiation resistance to enable their use as test organisms in radiation sterilization. The resistance of indicators prepared using these bacteria drops rapidly during storage, since both species are only vegetative cells. It is /551
possible that freeze-dried indicators of this type packed in gas-tight containers would show greater storage stability. It is also possible that the relatively good storage stability of bacterial spores could be utilized, although the effective resistance of the test objects would have to be increased to the required level by the addition of radiation protective substances.

Conclusion

Considering the logarithmic course of the survival curves, the limited efficacy of the sterilization processes and the unknown resistance of the population located on the goods to be sterilized, the requirement that the objects are freed of all viable organisms by sterilization cannot be fulfilled in most instances in the strictest sense of the word. In practice, sterilization yields an object, which -- with a certain, usually very high degree of probability -- does not harbor any organisms that represent an intolerable risk when the object is used for its intended purpose. The microbiologcial sterilization indicators serve to determine that the methods as such are capable of decimating a population of known resistance under known conditions and to a certain degree.

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